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Exploiting Gene Expression Kinetics in Conventional, Hyperfractionation, and Hypofractionation for Targeted Therapy

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Abstract

The dramatic changes in the technological delivery of radiation therapy, the repertoire of molecular targets for which pathway inhibitors are available, and the cellular and immunologic responses that can alter long-term clinical outcome provide a potentially unique role for using the radiation-inducible changes as therapeutic targets. Various mathematical models of dose and fractionation are extraordinarily useful in guiding treatment regimens. However, although the model may fit the clinical outcome, a deeper understanding of the molecular and cellular effect of the individual dose size and the adaptation to repeated exposure, called multifraction (MF) adaptation, may provide new therapeutic targets for use in combined modality treatments using radiochemotherapy and radioimmunotherapy. We discuss the potential of using different radiation doses and MF adaptation for targeting transcription factors, immune and inflammatory response, and cell “stemness.” Given the complex genetic composition of tumors before treatment and their adaptation to drug treatment, innovative combinations using both the pretreatment molecular data and also the MF-adaptive response to radiation may provide an important role for focused radiation therapy as an integral part of precision medicine and immunotherapy.

Introduction

Over the past 2 decades, the development of and innovations in “omics” technology provide a better understanding of the biological effects of radiation and chemotherapy in normal tissues and tumors, which has in turn led to the improvement in combined modality therapy.¹

Radiotherapy (RT) delivered in a fractionated regime is based on the differing radiobiological responses of cancer and normal tissues.^{2–4} Unlike tumor cells, normal cells repopulate during or shortly after a course of therapy, thus providing an opportunity for the repair of normal tissue damaged due to radiation. The difference in the shape of the radiation survival curve using various radiobiological models from clinical data helps to explain the

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clinical outcome from both dose size and fractionation scheme.^{5,6} Conventional RT (ConvRT) is administered in 1.8–2.2 Gy single fractions per day, 5 days per week for a total of 3–9 weeks, and maximum dose between 60 and 90 Gy.^{7–9} In contrast, hyperfractionated RT (HyperRT) is administered in smaller doses of 0.5–1.8 Gy with multiple fractions per day for 2–4 weeks, and hypofractionated RT (HypoRT) as single daily fractions 3–20 Gy with a small number of fractions usually over a week. Overall, advances in technology such as intensity-modulated RT, image-guided RT, stereotactic body RT, stereotactic radiosurgery, and protons and carbon RT have improved the ability to deliver higher radiation dose more accurately to tumors.^{3,5} With these technologies, there is still some dose heterogeneity, especially with intensity-modulated RT and image-guided RT, which often by design, include a greater volume of surrounding normal tissue around the target area receiving a smaller overall dose.^{1,3,10}

To date, a key determinant for selection of optimal fractionation schedules and dose is the site of tumor being treated. In comparison to ConvRT, HypoRT is the “new kid on the block.” There are currently several ongoing and a few complete randomized clinical trials evaluating patient survival, normal tissue effects (acute and late), and several other endpoints of HypoRT or HyperRT or all of these in comparison with ConvRT (Table 1). Recent Phase III reports by Lee et al⁸ and Wilkins et al⁹ show that HypoRT is not inferior to ConvRT in localized prostate cancer. Consequently, HypoRT could become the standard treatment for localized prostate cancer, as HypoRT may provide the benefit of shorter, potentially less costly, and more efficient treatment schedules and fewer patient visits. HyperRT, on the contrary, has been shown to be superior to ConvRT in head and neck cancer,^{11,12} small-cell lung cancer,¹³ and non-small cell lung cancer.^{14–16}

Although the effects of radiation on key cellular processes and the associated phenotypic changes (cell cycle arrest, cell death, and DNA repair) are well established in literature, knowledge of the different patterns in gene expression resulting from ConvRT, HyperRT, and HypoRT is limited. Understanding these differences could lead to selective induction of molecular targetable pathways that would enhance tumor killing, potentially using radiation in unique ways by which to improve overall patient outcomes. The use of molecular profiling of tumors treated with fractionated radiation could potentially improve patient outcomes by providing opportunities to select optimal radiation regimens and to allow for identification of targetable pathways and molecules altered because of fractionation. Preclinical evidence shows that fractionated radiation, with doses ranging from 0.5–30 Gy can induce an extensive array of targetable molecules (Table 2). These radiation-induced changes are based on the dose size and number of fractions.^{17–30} Selection of optimal fractionated radiation regimens and molecular-targeted therapies resulting from molecular tumor profiling could potentially improve patient outcomes. Other than with immunotherapy,^{31,32} there are currently no clinical reports of combined modality therapies exploiting fractionated radiation-induced molecular changes.

Current developments in genomic technology and molecular-targeted therapy allow for classification of patients as part of “precision medicine” and also elucidation of radiation-induced biological changes and selection of effective treatment strategies. The radiation-induced molecular phenotypes are influenced by a possible number of factors such as the

underlying genetic profile of the tumor—stage, site and hypoxic state of the tumor,³ type of radiation, and dose and fractionation schedule.^{10,33} Ongoing clinical trials may provide data that could potentially identify patient groups that would respond better to specific radiation regimens and combined modality therapies.

In this article, we examine targetable pathways and molecular-targeted therapies in the context of how fractionated radiation (conventional, hyperfractionation, and hypofractionation) regimes can be used to develop unique combined-modality strategies. Our discussion is based on the current literature of fractionated radiation-induced gene expression (Table 2) as follows: (1) transcription factors, NF- κ B and STAT1, (2) immune and inflammatory response, and (3) stem cellness and epithelial-to-mesenchymal transition (EMT).

Radiation and Transcription Factors

NF- κ B

As a transcription factor that regulates the expression of genes involved in cell differentiation or stemness,³⁴ cell proliferation, cell death, immune and inflammation responses,^{34–36} and the discussion of the role NF- κ B plays in cellular response to radiation is essential. Modulation of its expression or activation by radiation has been published in multiple reports.^{20,36–39} In 2009, Madhusoodhanan et al²⁰ showed increased NF- κ B activation after fractionated radiation. Poly(ADP-ribose) polymerase³⁹ and ataxia telangiectasia mutated,⁴⁰ proteins with well-established roles in DNA repair, have been reported to function as mediators of radiation-induced NF- κ B activation. Although NF- κ B has been associated with increased invasiveness, stemness and resistance to therapy, the clear dependence of the “Radiation Survivor”¹⁰ phenotype on its transcriptional activity²⁰ make it exploitable to combined modalities of radiation and targeted therapies.

Although it may be necessary to use NF- κ B inhibitors in chronic therapeutic strategies to enhance tumor cell killing, it should be noted that there is an ongoing discussion regarding the success of molecularly therapies targeting NF- κ B, as its prolonged inhibition could be immunosuppressive and compromise normal tissue homeostasis.^{41,42} Activity of NF- κ B proteins varies depending on the cell type, upstream stimuli, and the cell's microenvironment.⁴³ As a transcriptional activator, constitutively active NF- κ B primarily confers pro-survival, antiapoptotic, or proinflammatory properties or all of these in tumors.⁴³ Conversely, NF- κ B activation resulting from treatment with some DNA damaging agents have been reported to be transcriptionally repressive.⁴⁴ As reviewed by Chaturvedi et al⁴³ on NF- κ B activation in cancers, “one size does not fit all.” A well-defined mechanism for its constitutively or radiation-induced activation is necessary for the development of effective combined modality strategies that use radiation and molecular-targeted NF- κ B therapy. Alternatively, specific radiation-induced NF- κ B, coregulators, and other downstream effector pathways and molecules could also be targeted, as they are more likely to spare normal tissue and be specific to the tumors.^{45,46}

STAT1

STAT1 is a transcription factor that promotes the expression of genes involved in cellular differentiation, cell proliferation, and cell death. It is also an essential signaling molecule in response to interferon (IFN) stimulation. Tsai et al¹⁸ report the upregulation of STAT1 expression and other IFN-related genes after fractionated radiation. Our group has also reported a fractionated radiation-induced upregulation of STAT1 and IFN-inducible genes and proteins in primary human normal endothelial⁴⁷ and a prostate cancer model.^{22,25} Like NF- κ B, STAT1 and IFN γ -related genes have been implicated in chronic inflammation,¹⁸ increased resistance to therapy, and an aggressive invasion or metastatic phenotype.^{48–50}

Targeting Radiation-Induced Modulation of Immune Response and Inflammatory Response

The emerging role of fractionated radiation in eliciting an immune response cancer therapy has been reported by multiple groups and is the basis of many ongoing clinical trials.^{31,32,51} Published studies on the effect of radiation fractionation show an induction of a proimmunogenic gene expression profile.^{18,23–25} This correlates with the previously discussed upregulation of STAT1 and IFN γ -related genes. Dose per fraction has been shown to play a significant role in the observed immune-modulatory effects on fractionation.^{10,31,32} A better understanding of the gene expression resulting from fractionated radiation is necessary to take advantage of pathway changes and to optimize combined modalities that incorporate RT and immunotherapy.

Targeting Radiation-Induced Modulation of Stem Cellness and EMT

Cell surface markers for stem cellness such as CD133, CD24, and CD44 are well documented in subpopulations of cancer cells.⁵² Cells with phenotypic stem cell properties, cancer stem cells, have been associated with increased EMT^{52–54} and expression of WNT/ β -catenin^{55,56} and Notch^{57,58} signaling pathways. These pathways are essential to stem cell self-renewal, embryonic development, tissue differentiation, and maintenance of tissue homeostasis. Interactions between WNT/ β -catenin, Notch signaling, and EMT, are in network with other intersignaling and intrasignaling pathways that modulate the microenvironment, cell mobility, cell proliferation, and death. Cancer stem cells have been implicated in the development of chemoresistance and radioresistance,^{59,60} that ultimately contribute to recurrence, metastasis, and treatment failure.^{57,61,62} This association is not surprising, as the activation of WNT/ β -catenin and Notch signaling accelerates the cell proliferation, whereas an EMT phenotype significantly increases the invasive and metastatic potential of the cells.

As shown in Table 2, the resultant phenotype of increased EMT and stemness observed after fractionated radiation,^{26,30} provides extensive molecular targets that can be pursued to improve the efficacy of RT. However, a better understanding of these fractionated radiation-induced molecular changes is essential and cannot be overstated.

Conclusion

In clinical radiation therapy, the choice of fractionation scheme is based in large part on clinical experience and limited by normal tissue injury and employs mathematical models that are quite useful for clinical practice.² Although the mathematical models are useful, there may be unexplored benefits from studying the molecular and cellular effects of different doses and schedules, particularly when major alterations in therapy are undertaken as with the current use of hypofractionation. The articles by Brown et al,³ Shuryak et al,⁵ and McKenna et al⁶³ conclude that no additional tumoricidal mechanisms are needed to explain the tumor control at higher doses compared with lower doses. Firstly, an important caveat from clinical experience is that significant late effects may not occur for years or decades as seen with standard fractionation. The effect of large doses per fraction may take a while longer to elucidate. Secondly, in instances where mathematical models fit the data,^{5,63} there is no implication of molecular mechanisms. Although, these models^{3,5,63} suggest that no other distinct tumoricidal mechanisms need to be invoked, it is worth investigating the underlying mechanisms of radiation response and adaptation, as these may open up new approaches to treatment and combined modalities. The diversity of targets within a tumor—the tumor cell, tumor stem cell, vasculature, immune cells, inflammatory cells, and stroma—make it intriguing to seek various windows of dose and fractionation to be able to selectively affect one or the other process for a therapeutic advantage.

Despite the use of mathematical models for clinical practice and analysis by Shuryak et al⁵ who suggest that distinct tumoricidal mechanisms do not determine tumor control at high doses per fraction, there is clearly a different biological response at higher and lower doses that might be exploitable in clinical RT. Preclinical reports have shown the induction of targetable molecular profile by fractionated radiation.^{17–30} Current advances in molecular technologies provide us an opportunity to probe biological changes arising from different fractionation regimens. Bioinformatics tools such as ingenuity pathway analysis (QIAGEN, Redwood City, CA) and PANTHER classification system⁶⁴ allow for comprehensive analysis of complex biological pathways and interactions. This makes it an apt time to implement the molecular profiling of tumors during radiation treatment, to optimize selection and use of combined modality therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Study ID	Title	Site	Dose per Fraction (Gy)	Total Dose	# of Fractions
NCT00156052 ^(C)	Hypofractionated radiotherapy postlumpectomy in women with node negative breast cancer	Breast	HypoRT 2.66 ConvRT 2	42.50 50	16 25
NCT00062309 ^(C)	Comparison of 2 radiation therapy regimens in treating patients with stage II or stage III prostate cancer	Prostate	HypoRT 2.7 ConvRT 2	70.2 76	26 38
NCT00667888 ^(ANR)	A Phase III intensity radiotherapy dose escalation for prostate cancer using hypofractionation	Prostate	HypoRT 2.52 ConvRT 1.7	75.6 72	30 42
NCT00331773 ^(ANR)	Efficacy and rectal toxicity of hypofractionated radiation therapy with daily image guidance	Prostate	HypoRT 2.5 ConvRT 1.8	70 73.8	28 41
NCT01920789 ^(ANR)	Stereotactic precision and conventional radiotherapy evaluation	Non-small cell lung cancer	HypoRT 22 ConvRT 2	66 70	3 35
NCT00909818 ^(ANR)	Hypofractionated versus standard fractionated whole breast irradiation to patients with node negative breast cancer	Breast	HypoRT 2.6 ConvRT 2	40 50	15 25
NCT01014130 ^(ANR)	Hypofractionated radiotherapy (stereotactic) vs conventional radiotherapy for inoperable early stage I non-small cell lung cancer (NSCLC)	Non-small cell lung cancer	HypoRT 18 ConvRT 2	54 60-66	3 30-33
NCT01444820 ^(ANR)	Hypofractionated, dose escalation radiotherapy for high-risk adenocarcinoma of the prostate	Breast	HypoRT 2.72 ConvRT 3.5	68 76	25 22
NCT00793962 ^(R)	A Phase 3 randomized clinical trial of postmastectomy hypofractionation radiotherapy in high-risk breast cancer	Breast	HypoRT 2.9 ConvRT 2	43.5 50	15 25
NCT01413269 ^(R)	Randomized study of hypofractionated and Conventional fractionation radiotherapy after breast conservative surgery	Breast	HypoRT 2.9 ConvRT 2	43.5 50	15 25
NCT01459497 ^(R)	Hypofractionated Image-guided radiation therapy (IGRT) in patients with stage II and III non-small cell lung cancer	Non-small cell lung cancer	HypoRT 4 ConvRT 2	60 60-66	15 30-33
NCT02206230 ^(R)	Trial of hypofractionated radiation therapy for glioblastoma	Glioblastoma	HypoRT 3 ConvRT 2	60 60	20 30
NCT02332408 ^(R)	CyberKnife based hypofractionated radiotherapy for vertebral hemangiomas	Hemangiomas	HypoRT 5 ConvRT 2	25 36	5 18
NCT02474641 ^(R)	Hypofractionation with simultaneous integrated boost vs standard fractionation in early breast cancer	Breast	HypoRT 2-3 ConvRT 1.8-2	52.56-58.56 60.4-66.4	21-24 33-38

Study ID	Title	Site	Dose per Fraction (Gy)	Total Dose	# of Fractions
NCT02690636 ^(R)	Conventional vs hypofractionated radiotherapy in node positive breast cancer	Breast	HypoRT 2.66 ConvRT 2	42.56 50	16 25
NCT00778908 ^(C)	Late course accelerated hyperfractionated IMRT for locoregionally advanced nasopharyngeal carcinoma	Nasopharynx	HyperRT 1.8–2 ConvRT 1.8–2	55.8–70 54–60	31–35 30
NCT00002727 ^(ANR)	Radiation therapy in treating patients with stage II cancer of the vocal cord	head and neck cancer	HyperRT 1.2 ConvRT 2	79.2 70	66 35

ANR = trial active, not recruiting; C = complete trial; R = trial active, recruiting.

Table 2

Tissue Type/Experimental Model	Total Dose (Gy)	Fractionation Regimen	Changes in Gene Expression	Targeted Therapies	Reference
Breast cancer/in vitro	40	2 Gy, 2 days/10 weeks	Modulation of effector genes involve in cycle control, apoptosis, and DNA repair		Li et al ¹⁷
Breast cancer/in vitro	10	2 Gy, 5 days/1 week	Upregulation of STAT1 and interferon-related gene regulation		Tsai et al ¹⁸
Glioma/in vitro					
Prostate cancer/in vitro					
Prostate cancer/mouse xenograft					
Breast and colon cancer	27	1.8 Gy, 5 days/3 weeks	Induction of MDR-related genes (time dependent)		Botke et al ¹⁹
Breast cancer/in vitro	10	2 Gy, 5 days/1 week	Upregulation of genes involved in DNA repair, apoptosis, adhesion, angiogenesis, invasion/metastasis senescence, cell cycle signal transduction, and transcription control (NFkB)		Madhusoodhanan et al ²⁰
Prostate cancer/in vitro	10	1 Gy x2, 5 days/1 week	Modulation of immune response activating genes	Adjuvant immunotherapy agents	Aryankalayil et al ^{21,22}
Nasopharyngeal carcinoma	10	2 Gy, 5 days/1 week	Increased expression of antiapoptotic genes, and genes involved in tumor invasion, adhesion, metastasis and MDR phenotype (time dependent)		Ji et al ³⁰
Prostate cancer/in vitro	5	0.5 Gy x2, 5 days/1 week	p53-dependent modulation of DNA repair and cell cycle regulatory genes. Up regulation of immune response and interferon-related genes	Adjuvant immunotherapy agents	Simone II et al ²⁵
Head and neck squamous cell carcinoma	10	2 Gy, 5 days/1 week	Modulation of glycolysis-related genes		Goetze et al ²⁹
Normal human endothelial cells/in vitro	10	1 Gy x2, 5 days/1 week	Down-regulation of DNA repair, homologous recombination. Up regulation of p53-regulated genes, immune response, apoptosis, inflammatory response		Palayoor et al ²³
Pancreatic cancer/in vitro	10	2 Gy, 5 days/1 week	Up regulation in functional regulators of autophagy, EMT and stem cell-associated up regulation	Inhibitors of survivin, autophagosome	Aravindan et al ²⁶
Pancreatic cancer/ mouse xenograft	30	2 Gy, 5 days/3 weeks			Aravindan et al ²⁷
Glioblastoma/mouse xenograft	8	4 Gy, 1 day/2 weeks	Differential expression in NHEJ and HR DNA repair pathways	Inhibitors targeting MPS1, DNAPK, and TOP2A	Maachani ²⁸